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Description of a new species from Yunnan, China, within the genus *Trimeresurus* (Reptilia, Squamata, Viperidae) by integrating morphological and genetic evidence

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Abstract

In this study, we describe a green pit viper of the genus *Trimeresurus* Lacépède, 1804, from Yunnan Province, China, based on both morphological and molecular evidence. Morphologically, the new species can be distinguished from other congeners by a combination of the following characteristics: (1) dorsal body grass-green, ventral body yellowish green; (2) postocular stripe absent in females; (3) ventrolateral stripe white, present on the first dorsal scale rows in females; (4) iris golden yellow in females; (5) DSR 19/20–19–15 ($N = 2$), VEN 145–147 ($N = 2$), SC 55–60 ($N = 2$); (6) 10–11 cephalic scales; (7) first supralabial separated from the nasal scale by a distinct suture. Phylogenetic analysis based on the mitochondrial cyt *b*, ND4, 12S rRNA, and 16S rRNA gene fragments indicates that the new species is genetically divergent from all congeners (BPP 1.00/UFB 100), with uncorrected genetic distances of the mitochondrial gene cyt *b* ranging from 3.6% (*T. mayaae*) to 13.1% (*T. hageni*, *T. septentrionalis*, *T. guoi*, and *T. ayeyarwadyensis*) compared with its congeners.

Key Words

Morphology, molecular phylogeny, Southwestern China, taxonomy, *Trimeresurus liqibini* sp. nov.

Introduction

The Asian pit viper genus *Trimeresurus* Lacépède, 1804, is one of the most complex and species-rich genera of venomous snakes, encompassing terrestrial to arboreal species and being widely distributed throughout South and Southeast Asia (Gumprecht et al. 2004; Malhotra and Thorpe 2004; Vogel et al. 2004). The genus *Trimeresurus* is morphologically heterogeneous and has been divided into six subgenera: *Trimeresurus* Lacépède, 1804; *Himalayophis* Malhotra & Thorpe, 2004; *Parias* Gray, 1849; *Popeia* Malhotra & Thorpe, 2004; *Viridovipera* Malhotra & Thorpe, 2004; and *Sinovipera* Guo & Wang, 2011. Currently, at least 56 species are recognized within the genus *Trimeresurus* Lacépède, 1804 (Idiiatullina et al. 2024; Liang et al. 2025; Xu et al. 2025).

The subgenus *Viridovipera* Malhotra & Thorpe, 2004, is a group of green arboreal pit vipers within the *Trimeresurus* complex, characterized by two main morphological features: the first supralabial being completely distinct from the nasal scale and the presence of a short, strong, and spinose hemipenis (Gumprecht et al. 2004; Malhotra and Thorpe 2004; Mirza et al. 2023). Currently, eight recognized *Trimeresurus* species are assigned to the subge-

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nus *Viridovipera*, including *T. stejnegeri* Schmidt, 1925; *T. yunnanensis* Schmidt, 1925; *T. medoensis* Zhao, 1977; *T. vogeli* David, Vidal & Pauwels, 2001; *T. truongsonensis* Orlov, Ryabov, Bui & Ho, 2004; *T. mayaae* Rathee, Purkayastha, Lalremsanga, Dalal, Biakzuala, Muansanga & Mirza, 2022; *T. nujiang* Liang, Ding, Vogel, Chen & Wu, 2025; and *T. pretiosus* Xu, Nguyen, Wang, Zhang, Poyarkov, Wei, Vogel, Peng & Weng, 2025.

Yunnan Province is located in the southwest of China, spanning the Hengduan Mountains and the Yunnan–Guizhou Plateau and bordering Myanmar, Laos, and Vietnam. Due to its unique geographical configuration and complex climatic conditions, the region supports exceptionally rich biodiversity, with some species being endemic to the area (Yang and Rao 2008). At least three subgenera and eight species of the genus *Trimeresurus* are known from Yunnan Province, including *T. albolabris*, *T. stejnegeri*, *T. yunnanensis*, *T. popeiorum*, *T. caudornatus*, *T. guoi*, *T. lanna*, and *T. nujiang* (Gray 1842; Schmidt 1925; Chen et al. 2020; Chen et al. 2021; Idiiatullina et al. 2024; Nguyen et al. 2024; Liang et al. 2025). Among these, three species belong to the subgenus *Viridovipera*, namely *T. stejnegeri*, *T. yunnanensis*, and *T. nujiang*. With the continued discovery and description of new *Trimeresurus* species from Yunnan Province, species diversity in this region is likely to be substantially underestimated.

During several field surveys conducted in Yunnan Province in 2019 and 2020, two *Trimeresurus* (*Viridovipera*) specimens resembling *T. nujiang* and *T. yunnanensis* were collected from Kunming City, Yunnan Province, China. Subsequent morphological and molecular analyses revealed that these specimens represent an undescribed species distinct from *T. nujiang*, *T. yunnanensis*, and all other recognized congeners. Herein, this taxon is described as a new species.

Materials and methods

Sampling

Two individuals of the new species were collected from Qinglong Gorge, Kunming City, Yunnan Province, China. Specimens were fixed in 95% ethanol for 24 h and subsequently stored in 75% ethanol. The specimens were deposited at Guangxi Normal University (**GXNU**), Guangxi, China.

Morphologic analysis

Comparative materials examined are listed in Appendix 1. Comparative morphological data were obtained from the literature, including Schmidt (1925), Zhao et al. (1998), David et al. (2001a), David et al. (2001b), David et al. (2002), Orlov et al. (2004), Yang and Rao (2008), Guo et al. (2009), Rathee et al. (2022), Nguyen et al. (2025), Liang et al. (2025), and Xu et al. (2025).

Morphological terminology follows Vogel et al. (2004) and Nguyen et al. (2024). Measurements were taken with a slide caliper to the nearest 0.1 mm, except for body and tail lengths, which were measured to the nearest 1 mm using a tape ruler. Ventral scales were counted following Dowling (1951). Enlarged shield(s) anterior to the first ventral were regarded as preventral(s). Dorsal scale rows were recorded at one head length behind the head, at mid-body, and at one head length before the vent, respectively. Dorsal scale rows at mid-body were counted at the midpoint of the snout–vent length. Cephalic scales were counted along a straight line between the centers of the supraoculars.

The morphometric characters are as follows: **DEL** = distance between the lower eye margin and the edge of the lip; **ED** = eye diameter; **HH** = head height measured at the maximum height of the head; **HL** = head length measured from the snout tip to the angle of the jaw; **HW** = head width measured at the widest part of the head; **SVL** = snout–vent length; **TaL** = tail length; **TL** = total length; **TaL/TL** = ratio of tail length to total length. The scalation characters are as follows: **ASR** = anterior dorsal scale rows; **Cep** = cephalic scales; **DSR** = dorsal scale rows; **IIN** = number of scales separating the internasals; **IL** = infralabial scales; **MSR** = dorsal scale rows at mid-body; **PSR** = posterior dorsal scale rows; **SC** = subcaudal scales; **SL** = supralabial scales; **VEN** = ventral scales. Measurements for bilateral head characters are presented in left/right order. Sex was determined by examining the presence of hemipenes.

Molecular analysis

Genomic DNA was extracted from ethanol-preserved liver tissue using the TIANamp Marine Animals DNA Kit (TIANGEN Biotech). Four mitochondrial DNA (mtDNA) fragments, including cytochrome b (cyt *b*), NADH dehydrogenase subunit 4 (ND4), 12S ribosomal RNA gene (12S rRNA), and 16S ribosomal RNA gene (16S rRNA), were amplified using polymerase chain reaction (PCR). The amplification of cyt *b* was conducted using primers Gludg (5'-AACCACCGTTGTACATCAACT-3') and H16064 (5'-CTTTGGTTTACAAGAACAATGCTTTA-3'), following Palumbi (1996) and Simon et al. (1994). The primers used for ND4 were NADH4 (5'-CACCTATGACTACCAAAAGCTCATGTAGAAGC-3') and H12763V (5'-TTCTATCACTTGGATTTGCACCA-3'), following Arevalo et al. (1994). The 12S rRNA fragment was amplified using primers L1091 (5'-AAACTGGGATTAGATACCCCACTAT-3') and H1557 (5'-GTACACTTACCTTGTTACGACTT-3'), following Knight and Mindell (1993). For 16S rRNA, primers 16SP7 (5'-CGCCTGTTTACCAAAAACAT-3') and 16SP8 (5'-CCGGTCTGAACTCAGATCACGT-3') were used, as described by Simon et al. (1994). PCR amplification was performed in 25 µl reactions using the following cycling conditions: initial denaturation at 95 °C

for 2 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 48 °C (cyt *b*), 56 °C (ND4), 52 °C (12S rRNA), or 54 °C (16S rRNA) for 25 s, elongation at 72 °C for 15 s, and a final elongation step at 72 °C for 2 min. Sequencing was conducted by Beijing TsingKE Biotech Co., Ltd. (Chengdu, China). Sequence data were uploaded to GenBank, with the available accession numbers listed in Suppl. material 1: table S1. Sequencing primers were identical to the amplification primers.

Twenty-two *Trimeresurus* taxa were selected for molecular phylogenetic analyses. *Protobothrops elegans* (Gray) and *Azemiops feae* Boulenger were used as outgroups (Malhotra and Thorpe 2004; Chen et al. 2020) and used to root the phylogenetic trees. Sequences were assembled and aligned using MEGA 11.0 (Kumar et al. 2018), evaluated, and manually corrected when necessary. After combining the sequences and converting them to PHYLIP format using BioEdit (Alzohairy 2011), optimal models of sequence evolution for each partition, including genes and codons, were selected using Bayesian information criterion analysis in PartitionFinder 2.1.1 (Lanfear et al. 2017). Maximum likelihood (ML) analysis was conducted in RAxML v8.2.4 (Stamatakis 2014) under the best-fit model with 1,000 ultrafast bootstrap replicates (Felsenstein 1985), continuing until a correlation coefficient ≥ 0.99 was reached. Bayesian inference (BI) analysis was conducted using MrBayes 3.2 (Ronquist et al. 2012), in which two independent runs with four Markov chain Monte Carlo (MCMC) chains (three heated and one cold) were performed for 20 million generations and sampled every 1,000 generations. Uncorrected pairwise genetic distances (p-distances) among species based on cyt *b* sequences were calculated using MEGA 11.0 (Kumar et al. 2018).

Results

Phylogenetic analysis

Our dataset contained twenty-two *Trimeresurus* species, with the concatenated sequence alignment being 3,192 bp in length (cyt *b* = 1,074 bp; ND4 = 690 bp; 12S rRNA = 903 bp; 16S rRNA = 525 bp). The ML and BI analyses were conducted using RAxML v.8.2.4 and MrBayes 3.2, with the same models applied: GTR+I+G was the best fit for 12S rRNA, 16S rRNA, and the first codon positions of cyt *b* and ND4; HKY+I+G for the second codon positions of cyt *b* and ND4; and GTR+G for the third codon positions of cyt *b* and ND4.

Both ML and BI analyses recovered trees with consistent phylogenetic structure (Fig. 1). Phylogenetically, the two specimens from Kunming City, Yunnan Province (GXNU251216 and GXNU251217), form a clearly divergent lineage (Fig. 1; BPP 1.00/UFB 100 for BI and ML, respectively). These two specimens (GXNU251216 and GXNU251217) were recovered as the sister taxon to GP37 and GP38, collected from Huili, Sichuan Province, China, with high nodal support (Fig. 1; BPP 1.00/UFB 96). The uncorrected pairwise distances between the new species and other recognized *Trimeresurus* species range from 3.6% (*T. mayaae*) to 13.1% (*T. hageni*, *T. septentrionalis*, *T. guoi*, and *T. ayeyarwadyensis*), which are larger than those observed between some known species pairs (e.g., ≥ 3.6% between *T. nebularis* and *T. popeiorum*), indicating interspecific-level divergence (Suppl. material 1: table S2).

Because the topotypes (CIB DL2020090801, CIB DL30, GXNU2024071032, and GXNU2025010203) of *T. yunnanensis* were collected from Tengyueh (now Tengchong City), Yunnan Province, China (Schmidt 1925), and sequences from Yunnan Province (Jingdong County, Longling County, and Lincang City), northern Laos, the northern part of central Thailand, and northern and central Vietnam form a monophyletic group with strong support (Fig. 1; BPP 1.00/UFB 99), these specimens are treated as *T. yunnanensis*. However, twenty-three specimens from Yunnan Province (serial nos. 20–42, from Honghe, Jinping, Pingbian, and Yuanjiang counties, and Mengzi City) fall outside the *T. yunnanensis* clade. Deep divergences, both intraspecific (0.000–0.030; Suppl. material 1: table S2) and interspecies (≥ 0.045; Suppl. material 1: table S2), indicate that these specimens represent cryptic diversity. Therefore, these specimens are referred to as *T.* cf. *yunnanensis*, and their taxonomic status remains unresolved. Uncorrected sequence divergence in the cyt *b* gene fragment shows that the new species differs from *T. yunnanensis* by interspecific genetic distances of 0.071–0.085 and from *T.* cf. *yunnanensis* by genetic distances of 0.062–0.065 (Suppl. material 1: table S2).

Taxonomy

Trimeresurus liqibini **Liang, Ding, Wu, Yang & Chen, sp. nov.**

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Figs 2–4

Type material. ***Holotype*** • GXNU251216, adult female, collected from Qinglong Gorge, Kunming City, Yunnan Province, China (25.05676540°N, 102.37080426°E; elevation 1831 m a.s.l.), collected by Li Ding on 20 June 2020.

Paratype • GXNU251217, subadult female, collected in the same locality as the holotype by Li Ding on 28 May 2019. Morphological measurements are listed in Table 1.

Etymology. The specific name is in honor of Prof. Qibin Li (Guangxi, China) for his significant contributions to snakebite prevention, treatment, and the clinical application of snake venom. As a common name, we suggest "Li Qibin's Green Pit Viper" in English and "Kūn Míng Zhú Yè Qīng (昆明竹叶青)" in Chinese.

Diagnosis. *Trimeresurus liqibini* sp. nov. can be distinguished from all other congeneric species by a combination of the following morphological characters: (1) Dorsal body grass-green, ventral body yellowish-green. (2)

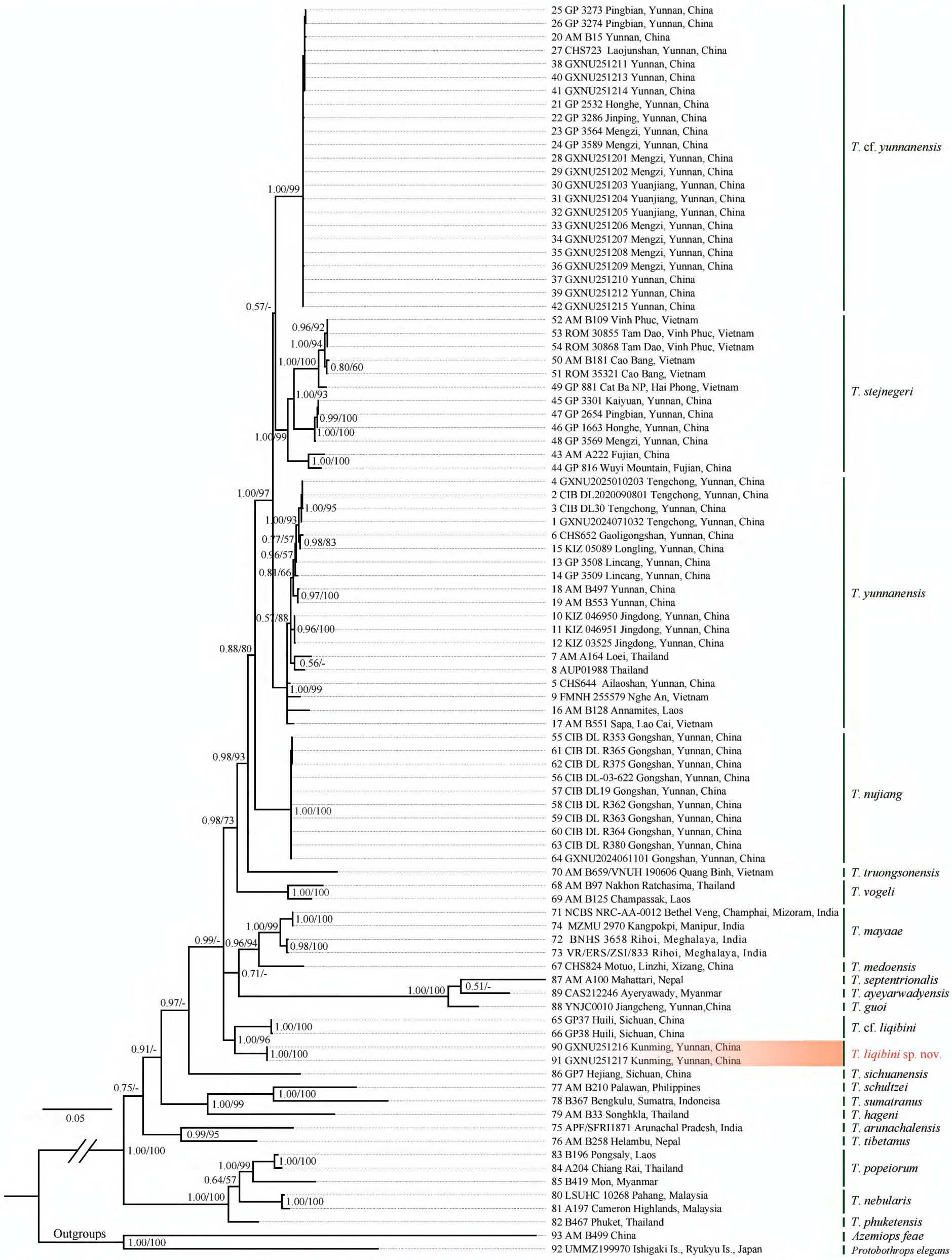

Figure 1. Phylogram of the genus *Trimeresurus* inferred from four mitochondrial (cyt *b*/ND4/12S rRNA/16S rRNA) gene fragments. Branch support values are presented as Bayesian posterior probabilities (BPP) / ultrafast bootstrap support (UFB). Values lower than 50 or 0.5 are shown as "–".

Lateral head grass-green above lower margin of eyes, and light green below, without postocular stripes in females. (3) Ventrolateral stripe white, present on outermost rows of dorsal scales in females. (4) Iris golden yellow in females. (5) First supralabial separated from nasal scale by a distinct suture. (6) Head scales feebly keeled; dorsal

Table 1. Measurements (to 0.1 mm) of the type specimens of *Trimeresurus liqibini* sp. nov.

Specimen voucher	GXNU251216	GXNU251217
Type	Holotype	Paratype
Sex	Female	Female
SVL (mm)	522	319
TaL (mm)	111	66
TL (mm)	633	385
TaL/TL	0.175	0.171
HL (mm)	29.6	19.6
HW (mm)	19.2	12.8
HH (mm)	10.1	9.1
DEL (mm)	3.75	2.74
ED (mm)	3.54	2.94
DSR	19–19–15	20–19–15
Pre-Ven	0	2
VEN	147	145
SC	60	55
VEN+SC	207	200
Cep	10	11
IIN	1	1
SL	9/10	10/11
IL	11/13	13/13
Iris color	Golden yellow	Golden yellow
Postocular stripe	Absent	Absent
Ventrolateral stripe	White	White

scale rows 19 (20)–19–15 ($N = 2$), slightly keeled except the outermost rows; ventral scales 145–147 in females ($N = 2$); subcaudal scales 55–60 in females ($N = 2$). (7) Tail prehensile, predominantly reddish-brown; tail moderate in length, with TaL/TL ratios of 0.171–0.175 in females. (8) Internasals not in contact, usually separated by one scale. (9) Supraoculars large but elongate, separated by 10–11 cephalic scales.

Description of the holotype (female) GXNU251216 (Figs 2–4).

Morphology. Body cylindrical and elongated (SVL 522 mm, TaL 111 mm, and TL 633 mm), tail moderate in length (TaL/TL 0.175) (Fig. 3). Head triangular in dorsal view (Fig. 4A), elongate, clearly distinct from neck (HL 29.6 mm; HW 19.2 mm; HW/HL 0.649). Snout elongated, flattened, round anteriorly in dorsal view (Fig. 4A), rather rectangular in lateral view (Fig. 4C) (DEL 3.75 mm). Medium sized eyes (ED 3.54 mm, ED/HL 0.120); the pupil vertically elliptical.

Body scalation. DSR 19–19–15, rhomboid, feebly keeled, gradually smoother towards ventral scales, the outermost rows smooth. VEN 147; SC 60, all paired, plus one terminal scale; anal shield entire; total number of VEN+SC 207.

Head scalation. Rostral trapezoidal when viewed from the front, lower margin of rostral nearly twice as wide as upper margin, height six sevenths of width of base, and obliquely truncated when viewed from the lateral side; nasal large, sub-rectangular, undivided, completely separated from the 1st supralabial by a suture behind the nostril; a pair of trapezoidal internasals significantly enlarged, separated from each other by a single triangular scale; nostril positioned centrally within the nasal

Figure 2. Holotype of *Trimeresurus liqibini* sp. nov. in life (adult female, GXNU251216).

Figure 3. Holotype of *Trimeresurus liqibini* sp. nov. in preservative (adult female, GXNU251216). **A.** Dorsal view of body; **B.** Ventral view of body.

scale; 3/3 preoculars elongated, present on each side of the head, two lower preoculars and the 2nd supralabial encompass the loreal pit which is triangular; 2/2 upper preoculars above the loreal pit, the upper one largest and visible from above, both scales elongate and in contact with loreal; lower preocular forming the lower margin of loreal pit; the 2nd supralabial completely contacts the anterior margin of the pit; 10 cephalic scales in a straight line between supraoculars, differing in size, all smooth, flat and juxtaposed; temporal and occipital scales feebly keeled; 9/10 supralabials; the 1st supralabial triangular, small; the 2nd supralabials tall, entirely bordering the anterior margin of the loreal pit, in contact with the nasal on both sides; the 3rd supralabial is longest and highest, in contact with the subocular; the 4th and 5th supralabials are separated from the subocular by one large scale on each side; the 6th supralabials are separated from subocular by 2/2 scales; 11/13 infralabials; the first pair of infralabials are longitudinally in contact with each other behind the mental; the first three pairs of infralabials are in contact with the anterior chin shields.

Coloration in life (Fig. 2). Description based on holotype GXNU251216. The dorsal surface of the head and body is uniformly grass-green, the lateral body mostly grass-green above and gradually lighter from spine to ventral, with a white ventrolateral stripe present on the first of DSR. Lateral head green above lower margin of eyes, and gradually yellowish-green below, without postocular stripes. Supralabials and infralabials yellowish-green edged with blue and molted with light cyan. Interstitial skin is predominantly black. The ventral surface is yellowish-green. Tail mostly reddish-brown, green laterally with a visible border between the colors. Eyes golden-yellow, with a blue edge from the snout to around the pupil.

Coloration in preservation (Figs 3, 4). Description is based on holotype GXNU251216, which is stored in 75% ethanol for about two years. The dorsal body is uniformly olive drab above and on the upper part of the sides and gradually turns yellowish-green on the lower sides, with a white ventrolateral stripe. Lateral head olive drab above lower margin of eye, and greyish-green below, without a postocular stripe. The color of the iris fades to gray white. Tail olive drab as the body anteriorly, the posterior 30% gradually rusty brown.

Comparison. The new species is assigned to the subgenus *Viridovipera* based on phylogenetic evidence and

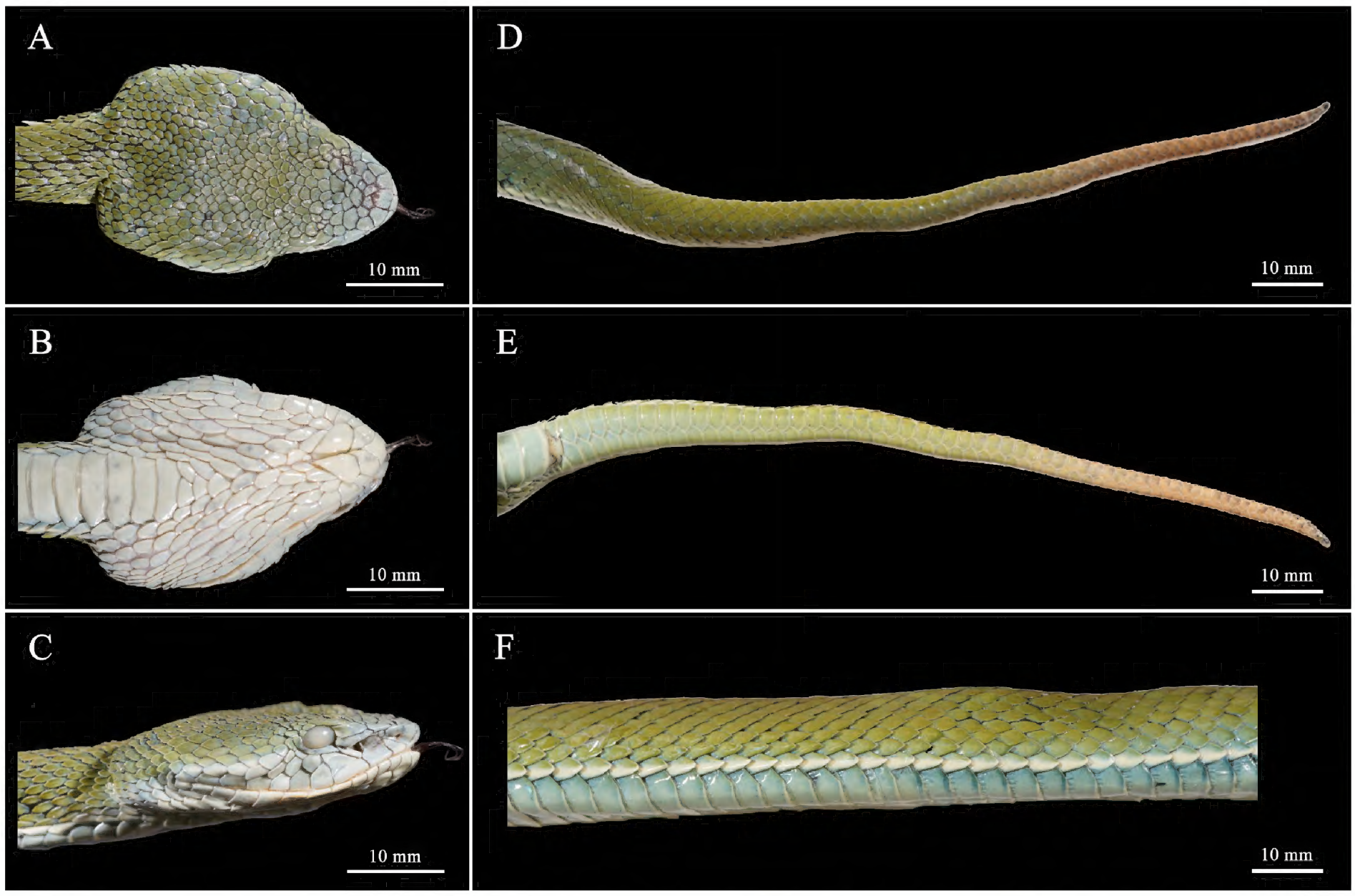

Figure 4. Holotype of *Trimeresurus liqibini* sp. nov. (adult female, GXNU251216). **A.** Dorsal view of head; **B.** Ventral view of head; **C.** Lateral view of head (right side); **D.** Dorsal view of tail; **E.** Ventral view of tail; **F.** Lateral view of the body (left side).

morphological characteristics (such as the first supralabials being completely separated from the nasal scale) (Malhotra and Thorpe 2004). The eight recognized species within the subgenus *Viridovipera* (*T. stejnegeri*, *T. yunnanensis*, *T. medoensis*, *T. vogeli*, *T. truongsonensis*, *T. mayaae*, *T. nujiang*, *T. pretiosus*) are regarded as the most relevant taxa for differential diagnosis. The main diagnostic characters distinguishing *Trimeresurus liqibini* sp. nov. from the other known members of the subgenus *Viridovipera* are (Suppl. material 1: table S3):

Trimeresurus liqibini sp. nov. closely resembles *T. nujiang* in morphology but differs from the latter by the following characters: (1) Smaller maximum SVL in females (522 mm vs. 682 mm); lower number of VEN in females (145–147 [146.0 ± 1.0] vs. 165–168 [166.6 ± 1.2]); lower total number of VEN+SC in females (200–207 [203.5 ± 3.5] vs. 222–226 [224.8 ± 1.6]). (2) Different color pattern in the outermost dorsal scale row in females. The upper and the lower parts of the first scale row are green, with a white ventrolateral stripe that occupies one-half of the outermost scale row in the middle vs. the lower third, and the upper portion of the first dorsal scale row yellowish-green and the middle third white in *T. nujiang*. (3) Different color pattern of the head, a blue edge present from the snout to around the pupil vs. a blue edge absent in *T. nujiang*.

Trimeresurus liqibini sp. nov. is similar to *T. yunnanensis* but can be distinguished from the latter by having: (1) Smaller body size, with the maximum SVL 522 mm in females vs. 1047 mm in *T. yunnanensis*; lower number of VEN in females (145–147 [146.0 ± 1.0] vs. 155–170 [160.6 ± 5.0]); lower total number of VEN+SC in females (200–207 [203.5 ± 3.5] vs. 209–232 [217.8 ± 6.1]). (2) Cephalic scales 10–11 vs. 9–12 (rarely 7 or 8) in *T. yunnanensis*.

Trimeresurus liqibini sp. nov. is distinct from *T. stejnegeri* by the following characters: (1) Smaller maximum SVL in females (522 mm vs. 627 mm); lower number of VEN in females (145–147 [146.0 ± 1.0] vs. 155–168 [162.6 ± 4.3]); lower number of SC in females (55–60 [57.5 ± 2.5] vs. 57–68 [61.1 ± 3.6]; lower total number of VEN+SC in females (200–207 [203.5 ± 3.5] vs. 212–231 [222.6 ± 6.9]). (2) Fewer dorsal scale rows at mid-body (MSR 19 vs. 21). (3) Eyes golden-yellow in females vs. yellow or amber in *T. stejnegeri*.

Trimeresurus liqibini sp. nov. is different from *T. medoensis* by the following characters: (1) Lower max SVL in females (522 mm vs. 555 mm). (2) MSR 19 vs. 17 in *T. medoensis*. (3) Higher number of cephalic scales, 10–11 vs. 6–9 (rarely 10) in *T. medoensis*. (4) The ventrolateral stripe is uniformly white in females, in contrast to the red (below) and white (above) or white stripe observed in *T. medoensis*. (5) Iris golden-yellow in females vs. yellow or yellowish-green in *T. medoensis*.

Trimeresurus liqibini sp. nov. differs from *T. vogeli* by having: (1) Lower max SVL in females (522 mm vs. 947 mm); lower number of VEN in females (145–147 [146.0 ± 1.0] vs. 157–173 [166.3 ± 6.4]); slightly lower number of SC in females (55–60 [57.5 ± 2.5] vs. 59–65 [60.8 ± 2.0]); lower number of VEN+SC in females

(200–207 [203.5 ± 3.5] vs. 218–233 [227.2 ± 5.5]). (2) Fewer dorsal scale rows at mid-body (19 vs. 21, rarely 20). (3) Lower number of cephalic scales (10–11 vs. 11–14). (4) Iris golden-yellow in females vs. yellow or yellowish-green in *T. vogeli*. (5) Tail mostly reddish-brown in life vs. mostly green in *T. vogeli*.

Trimeresurus liqibini sp. nov. differs from *T. truongsonensis* by having: (1) Higher max SVL in females (522 mm vs. 462 mm); lower number of VEN in females (145–147 vs. 165); lower number of SC in females (55–60 vs. 70); lower total number of VEN+SC in females (200–207 vs. 235). (2) MSR 19 vs. 21 in *T. truongsonensis*. (3) Fewer cephalic scales, 10–11 vs. 10–13 in *T. truongsonensis*. (4) Blotches, spots, bands, or crossbar absent in dorsum vs. present in *T. truongsonensis*.

Trimeresurus liqibini sp. nov. can be distinguished from *T. mayaae* by having: (1) A relatively small body size, with a maximum known SVL of 522 mm in females (vs. 590 mm in *T. mayaae*). (2) Lower number of VEN in females (145–147 vs. 153); slightly higher number of SC in females (55–60 [57.5 ± 2.5] vs. 54–55 [54.5 ± 0.5]); slightly lower number of VEN+SC in females (200–207 [203.5 ± 3.5] vs. 207–208 [207.5 ± 0.5]). (3) 19 dorsal scales at mid-body, weakly keeled vs. MSR 21 (rarely 19/20), moderately keeled in *T. mayaae*. (4) Postocular stripe absent in females vs. a faint white postocular stripe present or absent in *T. mayaae*. (5) Irises golden yellow in females vs. green eyes in *T. mayaae*.

Trimeresurus liqibini sp. nov. can be distinguished from *T. pretiosus* by having: (1) Higher max SVL in females (522 mm vs. 512 mm); higher number of VEN in females (145–147 vs. 142); higher number of SC in females (55–60 vs. 54); higher total number of VEN+SC in females (200–207 vs. 196). (2) Subcaudal scales all paired vs. partially arranged in a single row in *T. pretiosus*. (3) Females have golden yellow irises, distinctly different from the orange-yellow eyes of *T. pretiosus*.

Distribution and habitat. *Trimeresurus liqibini* sp. nov. is currently known only from the type locality and its adjacent areas, which were found in the central Yunnan Plateau, at elevations reaching approximately 1,800 m in Kunming, Yunnan Province, China (Fig. 5). It is found in a typical subtropical mountain evergreen broad-leaved forest and deciduous broad-leaved mixed forest ecosystem, surrounded by karst landforms and humid climate, with high vegetation coverage and complex and diverse hillside habitats. In its typical locality, this species prefers to perch on branches, waiting for prey (Fig. 6).

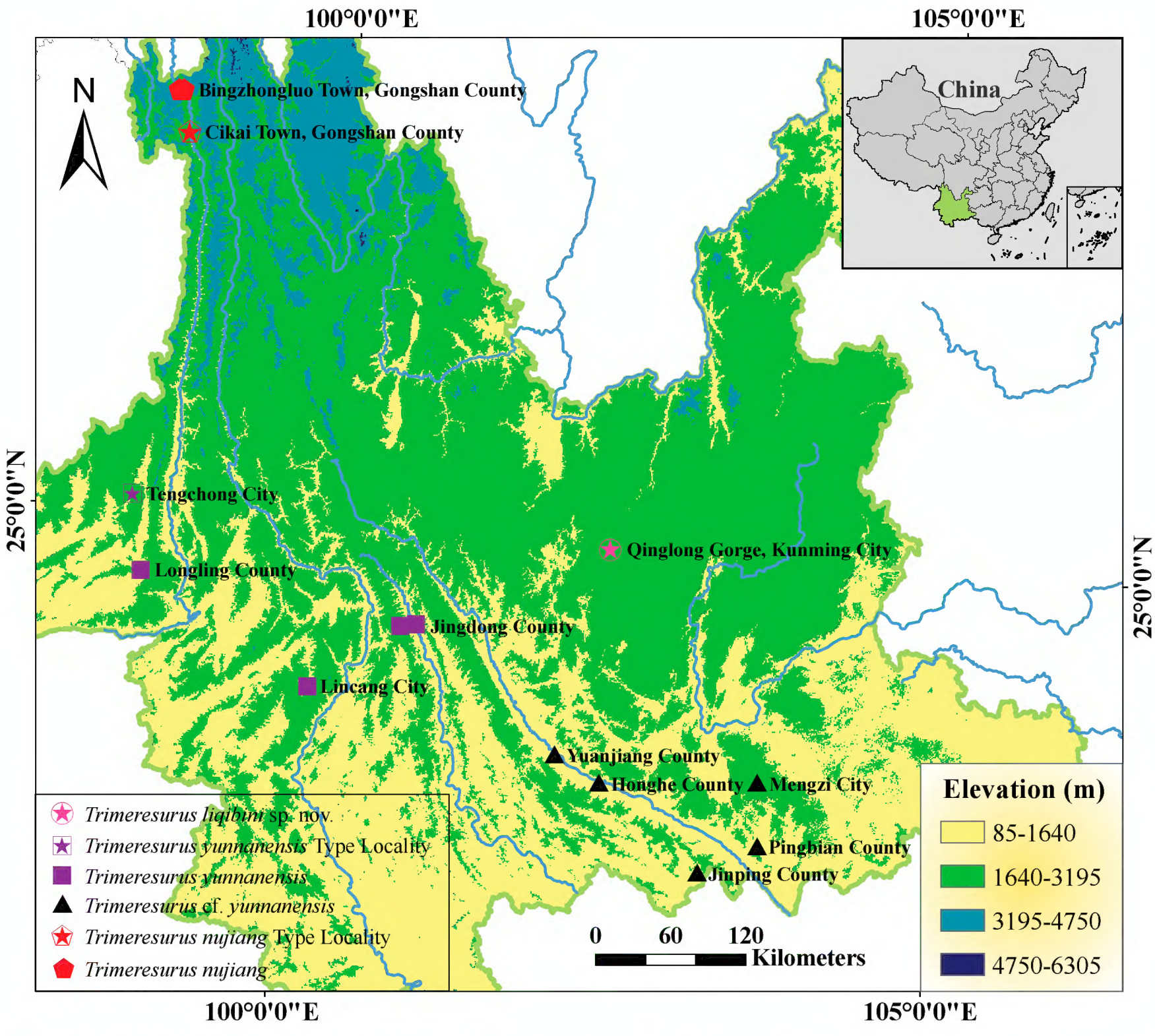

Figure 5. Geographical distribution of *T. liqibini* sp. nov., *T. yunnanensis*, *T.* cf. *yunnanensis*, and *T. nujiang* in Yunnan Province, China.

Figure 6. Macrohabitat of *Trimeresurus liqibini* sp. nov. in Qinglong Gorge, Kunming City, Yunnan Province, China.

Discussion

Guo et al. (2009) identified two *Trimeresurus* specimens from Huili City, Sichuan Province, China, as *T. yunnanensis* based on molecular and morphometric analyses. Based on molecular and morphological data, Liang et al. (2025) confirmed *T. gumprechti* David et al., 2002, as a subjective junior synonym of *T. yunnanensis* Schmidt, 1925, and proposed that two specimens from Huili City, Sichuan Province, should not be assigned to *T. yunnanensis* but instead represent a new undescribed species of the subgenus *Viridovipera*. In this study, the phylogenetic tree indicates that the new species forms a strongly supported clade with two specimens (GP37 and GP38) from Huili City, Sichuan Province, China. From a molecular phylogenetic perspective, in which named taxa are monophyletic, the new species represents an independent lineage, or, alternatively, may be conspecific with GP37 and GP38. However, due to the lack of available morphological data for specimens from Huili, Sichuan Province, their precise taxonomic status remains undetermined. Based on molecular and morphological evidence, we describe the population from Kunming City, Yunnan Province, China, as a new species. The population from Huili, Sichuan Province, is provisionally regarded as belonging to the *T. liqibini* complex. Future studies should prioritize increasing sample sizes for both this newly described species and the Huili population, with particular emphasis on obtaining and describing male specimens for accurate identification. Phylogenetic results also indicate that the twenty-three specimens from southern Yunnan (Honghe, Jinping, Pingbian, and Yuanjiang counties, and Mengzi City), previously identified as *T. gumprechti*, form a distinct evolutionary lineage and exhibit considerable genetic divergence from other congeners. Therefore, these specimens are referred to as *T.* cf. *yunnanensis*, and their formal taxonomic status requires further investigation. Additional sampling and integrative taxonomic assessments combining molecular and morphological data are likely to reveal further undescribed species.

Early classification efforts primarily relied on observational descriptions of external morphology (such as scale arrangement and body coloration patterns) and internal structures (such as skeletal structure and dental characteristics). However, certain morphological traits exhibit evolutionary conservatism among species within the same genus, and reliance solely on morphological data may lead to subjective misjudgments that underestimate species diversity. *T. yunnanensis* was diagnosed mainly on the basis of having 19 dorsal scale rows at mid-body (Schmidt 1925). Guo et al. (2009) proposed that dorsal scale rows at mid-body play a vital role in distinguishing *T. yunnanensis* from its congeners and are more reliably assessed by examining the body position at which the reduction from 21 to 19 scale rows occurs. Due to limitations in early taxonomy, specimens resembling *T. stejnegeri* and having 19 dorsal scale rows at mid-body were broadly classified as *T. yunnanensis* (Zhao et al. 1998; Guo et al. 2009; Liang et al. 2025). Based on integrated morphological and molecular data, Liang et al. (2025) recently described the population from Gongshan, Yunnan

Province, as *T. nujiang*. This highlights the necessity of an integrative approach combining morphological analysis, molecular evidence, and hemipenial examination for accurate species identification.

The description of *Trimeresurus liqibini* sp. nov. raises the total number of recognized *Trimeresurus* species to 57, of which 16 species have been reported from China. Its discovery further enhances knowledge of the biodiversity of Yunnan Province, increasing the number of recognized species within the subgenus *Viridovipera* to nine. Currently, three species of the subgenus *Viridovipera* are known from Yunnan Province, China, namely *T. yunnanensis*, *T. nujiang*, and *T. liqibini*. Despite recent progress in the taxonomy of *Viridovipera* in Yunnan, Southwestern China, knowledge of the diversity and distribution of these species remains incomplete. A substantial proportion of reported snakebite cases in Yunnan are caused by members of *Trimeresurus*, and their medical importance is significant. Therefore, future research should focus on the taxonomy, distribution, ecology, and toxicology of *Trimeresurus* pit vipers in Southwestern China, particularly their distributional ranges, habitat preferences, and population sizes within *T. yunnanensis*, *T.* cf. *yunnanensis*, *T. nujiang*, and *T. liqibini*. Continued field surveys across Yunnan Province and adjacent regions of Myanmar, Laos, and Vietnam are essential to clarify ecological niche breadth, overlap, and differentiation within the subgenus *Viridovipera* and to strengthen conservation planning for this biogeographically important region of the Hengduan Mountains and the Yunnan–Guizhou Plateau.

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Appendix 1

Comparative material examined:

Trimeresurus stejnegeri (*N* = 30). China. CIB DL2018090201, CIB DL2019040401, CIB DL2019092504, "Wuyi Mountain, Fujian". NHMUK 1940.3.19.21 "Fuzhou, Fujian". NHMUK 1899.4.24.61 "Guadun, Fujian". MH 010 "Minhou, Fujian". ZMH R07998 "Fujian". SMF 21223 "Hainan". FMNH 7134 "Anwei". NHMW 23913:1 "Daolin, Hunan". MH 002 "Sichuan". ZMH R07984 "Morisson Mountain, Taiwan". ZMH R07985–6, "Pingtung, Taiwan". NHMW 23906:2–6, "Tsu-Shari, Taiwan". NHMW 23907:1–4, "Jiayi, Taiwan". ZMH R07982 "Taiwan". ZMH R07983 "Candidius Sea, Taiwan". NHMW 23906:1, NHMW 23906:7, "Tsu-Shari, Taiwan". NHMW 23907:5–7, "China".

Trimeresurus yunnanensis (*N* = 11). China. FMNH 7064–5 (Paratypes), CIB DL30, CIB DL2020090801, GXNU 2024071032, NHMUK 1907.5.4.5, "Tengchong, Yunnan". Myanmar. CAS 241182 "Moenyin, Kachin". NHMW 23913:4 "Myanmar". Vietnam. IEBR 3918 HB.2014.31 "Hang Kia-Pa Co, Hoa Binh, Vietnam". Thailand. ZFMK 70444, ZFMK 78730, "Phu Luang".

Trimeresurus medoensis (*N* = 6). China. CIB 2015101601–2, "Motuo, Xizang". Myanmar. NHMUK 1936.7.4.43, NHMUK 1940.6.5.73, "Nam Ti Valley, Upper Burma". CAS 221528, NHMUK 1940.6.5.73, "Kachin".

Trimeresurus vogeli (*N* = 12). Vietnam. ZMH R09625–6, "Saigon now Ho Chi Minh City". ZFMK 86455–6, "Phong Nha Ke Bang NP, Quang Binh". ZFMK 91074–5, "Biduop-Nui Ba NP, Lam Dong". ZFMK 94923, ZFMK 92793–5, "Dak Lak, Chu Yang Sin NP". ZFMK 94275 "Ba To, Quang Ngai". Thailand. NHMUK 1937.2.1.38 "Hup Bon, Si Racha, Chonburi".

Trimeresurus truongsonensis (*N* = 1). Vietnam. VNMN 3020 "Phong Nha-Ke Bang NP, Quang Binh".

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Trimeresurus nujiang (*N* = 11). China. CIB DL R353 "Cikai, Gongshan, Yunnan". CIB DL10, CIB DL19, CIB DL R364, CIB DL R375, CIB DL R380, CIB DL-03-622, GXNU2024061101, "Gongshan County, Yunnan". CIB DL R362–3, CIB DL R365, "Bingzhongluo Town, Gongshan County, Yunnan".

Trimeresurus liqibini sp. nov. (*N* = 2). China. GXNU251216–7 "Qinglong Gorge, Kunming, Yunnan".

Supplementary material 1

Supplementary tables

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Data type: xlsx

Explanation note: **table S1.** Sequences and voucher specimens of the genus *Trimeresurus* and outgroup taxa used in this study. **table S2.** Uncorrected pairwise distances (percentage) between *Trimeresurus* species based on 1074 base pairs from the mitochondrial gene cyt *b*. **table S3.** Comparison of morphological characters in members of the subgenus *Viridovipera*.

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